CLAIMS

What is claimed is:

1. A method of increasing soluble levels of a heterologous protein expressed in a non-natural host by selective translational attenuation, the method comprising:

a) synthesizing a translationally-harmonized nucleic acid comprising a coding region consisting of at least 60 contiguous codons and having a nucleotide sequence at least 65% identical to a contiguous codon sequence native to a natural host wherein the coding region is translationally-harmonized by substituting each of at least 3 codons of the native contiguous codon sequence with a synonymous codon that has a lower usage frequency in the non-natural host, where the codons being replaced;

(i) are within 10 codons of a codon in the native codon sequence that has a low or intermediate codon usage frequency in the natural host; and,

(ii) are of intermediate or high usage frequency in the non-natural host;

b) introducing the harmonized nucleic acid into the non-natural host cell to generate a recombinant host; and,

c) culturing the non-natural host under conditions that permit the soluble level of the heterologous protein to exceed by more than 10% the soluble level of that protein when expressed from a non-harmonized nucleic acid in the non-natural host.

2. The method of claim 1, wherein the nucleotide sequence of the 60 contiguous codons is at least 85% identical to the nucleotide sequence of the native contiguous codon sequence.

3. The method of claim 1, wherein the coding region is translationally-harmonized by substituting each of at least 6 codons of the native contiguous codon sequence with a synonymous codon of the non-natural host.

4. The method of claim 1, wherein the codons being replaced are within 6 codons of a codon in the native codon sequence that has a low or intermediate codon usage frequency in the natural host.

5. The method of claim 1, wherein the codons being replaced are within 3 codons of a codon in the native codon sequence that has a low or intermediate codon usage frequency in the natural host.

6. The method of claim 1, wherein the translationally-harmonized nucleic acid further comprises expression control sequences operably-linked to the coding region.

7. The method of claim 1, wherein the heterologous protein is a fusion protein and the translationally-harmonized nucleic acid further comprises a second coding region in-frame with the first coding region.

8. The method of claim 7, wherein the translationally-harmonized nucleic acid further comprises a nucleotide sequence encoding a peptide linker in-frame with and located between the coding region and the second coding region.

9. The method of claim 7, wherein the second coding region encodes an affinity tag.

10. The method of claim 7, wherein the second coding region is translationally harmonized.

11. The method of claim 1, wherein the non-natural host is a bacterial cell.

12. The method of claim 1, wherein the non-natural host is a fungal cell.

13. The method of claim 1, wherein the non-natural host is an insect cell.

14. The method of claim 1, wherein the non-natural host is a plant cell.

15. The method of claim 1, wherein the natural host is a mammalian tissue cell and the non-natural host is a different mammalian tissue cell.

16. The method of claim 1, wherein the natural host is a first cell normally residing in a first mammalian species and the non-natural host is a second cell normally residing in a second mammalian species.

17. The method of claim 16, wherein the first cell and the second cell are from the same tissue type selected from the group consisting of liver, spleen, lymphoid, smooth muscle, striated muscle, nerve tissue and adipose tissue.

18. The method of claim 1, wherein the coding region encodes a mammalian protein.

19. The method of claim 18, wherein the coding region encodes a mammalian hormone.

20. The method of claim 1, wherein the coding region encodes a neuropeptide.

21. The method of claim 1, wherein the coding region encodes an antibody.

22. The method of claim 1, wherein the coding region encodes an antimetabolite.

23. The method of claim 1, wherein the coding region encodes an antibiotic.

24. The method of claim 1, further comprising isolating the heterologous protein.

25. The method of claim 24, wherein the heterologous protein further comprises a secretory sequence and isolating the heterologous protein comprises centrifuging the non-natural host culture.

26. A translationally-harmonized nucleic acid comprising a coding region for a recombinant protein, the coding region consisting of at least 60 contiguous codons and having a nucleotide sequence at least 65% identical to a contiguous codon sequence native to a natural host wherein the coding region is translationally-harmonized by substituting each of at least 3 codons of the native contiguous codon sequence with a synonymous codon that has a lower usage frequency in the non-natural host, where the codons being replaced;

(a) are within 10 codons of a codon in the native codon sequence that has a low or intermediate codon usage frequency in the natural host; and,

(b) are of intermediate or high usage frequency in the non-natural host.

27. The nucleic acid of claim 26, wherein the nucleotide sequence of the 60 contiguous codons is at least 85% identical to the nucleotide sequence of the native contiguous codon sequence.

28. The nucleic acid of claim 26, wherein the coding region is translationally-harmonized by substituting each of at least 6 codons of the native contiguous codon sequence with a synonymous codon of the non-natural host.

29. The nucleic acid of claim 26, wherein the codons being replaced are within 6 codons of a codon in the native codon sequence that has a low or intermediate codon usage frequency in the natural host.

30. The nucleic acid of claim 26, wherein the codons being replaced are within 3 codons of a codon in the native codon sequence that has a low or intermediate codon usage frequency in the natural host.

31. The nucleic acid of claim 26, further comprising expression control sequences operably-linked to the coding region.

32. The nucleic acid of claim 26, wherein the heterologous protein is a fusion protein and the translationally-harmonized nucleic acid further comprises a second coding region in-frame with the first coding region.

33. The nucleic acid of claim 32, further comprising a nucleotide sequence encoding a peptide linker in-frame with and located between the coding region and the second coding region.

34. The nucleic acid of claim 32, wherein the second coding region encodes an affinity tag.

35. The nucleic acid of claim 32, wherein the second coding region is translationally harmonized.

36. The nucleic acid of claim 26, wherein the non-natural host is a bacterial cell.

37. The nucleic acid of claim 26, wherein the non-natural host is a fungal cell.

38. The nucleic acid of claim 26, wherein the non-natural host is an insect cell.

39. The nucleic acid of claim 26, wherein the non-natural host is a plant cell.

40. The nucleic acid of claim 26, wherein the natural host is a mammalian tissue cell and the non-natural host is a different mammalian tissue cell.

41. The nucleic acid of claim 26, wherein the natural host is a first cell normally residing in a first mammalian species and the non-natural host is a second cell normally residing in a second mammalian species.

42. The nucleic acid of claim 41, wherein the first cell and the second cell are from the same tissue type selected from the group consisting of liver, spleen, lymphoid, smooth muscle, striated muscle, nerve tissue and adipose tissue.

43. The nucleic acid of claim 26, wherein the coding region encodes a mammalian protein.

44. The nucleic acid of claim 43, wherein the coding region encodes a mammalian hormone.

45. The nucleic acid of claim 26, wherein the coding region encodes a neuropeptide.

46. The nucleic acid of claim 26, wherein the coding region encodes an antibody.

47. The nucleic acid of claim 26, wherein the coding region encodes an antimetabolite.

48. The nucleic acid of claim 26, wherein the coding region encodes an antibiotic.

49. A recombinant cell comprising an expression system, including a translationally-harmonized nucleic acid comprising a coding region for a recombinant protein, the coding region consisting of at least 60 contiguous codons and having a nucleotide sequence at least 65% identical to a contiguous codon sequence native to a natural host wherein the coding region is translationally-harmonized by substituting each of at least 3 codons of the native contiguous codon sequence with a synonymous codon that has a lower usage frequency in the recombinant cell, where the codons being replaced;

(a) are within 10 codons of a codon in the native codon sequence that has a low or intermediate codon usage frequency in the natural host; and,

(b) are of intermediate or high usage frequency in the recombinant cell;

wherein the recombinant cell produces a soluble level of the heterologous protein exceeding the soluble level of that protein when expressed from a non-harmonized nucleic acid in the recombinant cell by more than 10%.

50. The recombinant cell of claim 49, wherein the nucleotide sequence of the 60 contiguous codons is at least 85% identical to the nucleotide sequence of the native contiguous codon sequence.

51. The recombinant cell of claim 49, wherein the coding region is translationally-harmonized by substituting each of at least 6 codons of the native contiguous codon sequence with a synonymous codon of the recombinant cell.

52. The recombinant cell of claim 49, wherein the codons being replaced are within 6 codons of a codon in the native codon sequence that has a low or intermediate codon usage frequency in the natural host.

53. The recombinant cell of claim 49, wherein the codons being replaced are within 3 codons of a codon in the native codon sequence that has a low or intermediate codon usage frequency in the natural host.

54. The recombinant cell of claim 49, wherein the translationally-harmonized nucleic acid further comprises expression control sequences operably-linked to the coding region.

55. The recombinant cell of claim 49, wherein the heterologous protein is a fusion protein and the translationally-harmonized nucleic acid further comprises a second coding region in-frame with the first coding region.

56. The recombinant cell of claim 55, wherein the second coding region encodes an affinity tag.

57. The recombinant cell of claim 55, wherein the second coding region is translationally harmonized.

58. The recombinant cell of claim 55, wherein the translationally-harmonized nucleic acid further comprises a nucleotide sequence encoding a peptide

linker in-frame with and located between the coding region and the second coding region.

59. The recombinant cell of claim 49, wherein the recombinant cell is a bacterial cell.

60. The recombinant cell of claim 49, wherein the recombinant cell is a fungal cell.

61. The recombinant cell of claim 49, wherein the recombinant cell is an insect cell.

62. The recombinant cell of claim 49, wherein the recombinant cell is a plant cell.

63. The recombinant cell of claim 49, wherein the natural host is a mammalian tissue cell and the recombinant cell is a different mammalian tissue cell.

64. The recombinant cell of claim 49, wherein the natural host is a first cell normally residing in a first mammalian species and the recombinant cell is a second cell normally residing in a second mammalian species.

65. The recombinant cell of claim 64, wherein the first cell and the second cell are from the same tissue type selected from the group consisting of liver, spleen, lymphoid, smooth muscle, striated muscle, nerve tissue and adipose tissue.

66. The recombinant cell of claim 49, wherein the coding region encodes a mammalian protein.

67. The recombinant cell of claim 66, wherein the coding region encodes a mammalian hormone.

68. The recombinant cell of claim 49, wherein the coding region encodes a neuropeptide.

69. The recombinant cell of claim 49, wherein the coding region encodes an antibody.

70. The recombinant cell of claim 49, wherein the coding region encodes an antimetabolite.

71. The recombinant cell of claim 49, wherein the coding region encodes an antibiotic.

72. A computer readable medium comprising:

a) code for a first set of instructions for identifying at least three codons in a first set of at least 60 statically ordered codons, wherein the identified codons have a low or intermediate codon usage in a first codon usage data set;

b) code for a second set of instructions for substituting each of the identified codons with a synonymous codon having a low or intermediate codon usage in a second codon usage data set, thereby generating a second set of at least 60 statically ordered codons;

wherein the first set of at least 60 codons is representative of a nucleic acid sequence of a natural host; the first codon usage data set contains data identifying the frequency of codon usage in expressed nucleic acid transcripts of the natural host for each synonymous codon corresponding to each natural amino acid; the second codon usage data set contains data identifying the frequency of codon usage in expressed nucleic acid transcripts of a non-natural host for each synonymous codon corresponding to each natural amino acid; and, the second set of at least 60 codons is representative of a nucleic acid translationally-harmonized for expression in the non-natural host.

73. The computer readable medium of claim 72, wherein the first set of instructions identify at least three codons in a first set of at least 60 statically ordered codons.

74. The computer readable medium of claim 72, wherein the non-natural host is a bacterial cell.

75. The computer readable medium of claim 72, wherein the non-natural host is a fungal cell.

76. The computer readable medium of claim 72, wherein the non-natural host is an insect cell.

77. The computer readable medium of claim 72, wherein the non-natural host is a plant cell.

78. The computer readable medium of claim 72, wherein the natural host is a mammalian tissue cell and the non-natural host is a different mammalian tissue cell.

79. The computer readable medium of claim 72, wherein the natural host is a first cell normally residing in a first mammalian species and the non-natural host is a second cell normally residing in a second mammalian species.

80. The computer readable medium of claim 79, wherein the first cell and the second cell are from the same tissue type selected from the group consisting of liver, spleen, lymphoid, smooth muscle, striated muscle, nerve tissue and adipose tissue.

81. The computer readable medium of claim 72, wherein the at least 60 statically ordered codons encodes a mammalian protein.

82. The computer readable medium of claim 81, wherein the at least 60 statically ordered codons encodes a mammalian hormone.

83. The computer readable medium of claim 72, wherein the at least 60 statically ordered codons encodes a neuropeptide.

84. The computer readable medium of claim 72, wherein the at least 60 statically ordered codons encodes an antibody.

85. The computer readable medium of claim 72, wherein the at least 60 statically ordered codons encodes an antimetabolite.

86. The computer readable medium of claim 72, wherein the at least 60 statically ordered codons encodes an antibiotic.